

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of: Jesper Vind Confirmation No: To be assigned

Serial No.: To be assigned Group Art Unit: To be assigned

Filed: December 5, 2001 Examiner: To be assigned

For: Method For Producing a Polynucleotide Library

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, DC 20231

Sir:

Before examination, please amend the above-identified application as follows (a marked up version pursuant to 37 C.F.R. 1.21 is attached hereto):

**IN THE SPECIFICATION:**

**IN THE CLAIMS:**

Please cancel claims 19 and 20, without prejudice of disclaimer:

Please substitute the following amended claims for the pending claims having the same claim numbers:

4. (Amended.) The method of claim 1, wherein the at least two homologous double-stranded template polynucleotides encode homologous enzymes, preferably amylases, proteases, cellulases, lipases, xylanases, or phospholipases.

5. (Amended.) The method of claim 1, wherein the solution comprises a population of cells or a lysate of a population of cells.

7. (Amended.) The method of claim 5, wherein the population of cells or the lysate of a population of cells comprises the mismatch repair protein(s).

8. (Amended.) The method of claim 5, wherein the population of cells, or the population of cells giving rise to the lysate, do not methylate newly synthesized polynucleotides.

9. (Amended.) The method of claim 1, wherein the mismatch repair protein(s) is (are) thermostable.

10. (Amended.) The method of claim 1, wherein the thermostable mismatch repair protein(s) comprises a MutS homologue, preferably MutS YT1 of *Thermus aquaticus*.

11. (Amended.) The method of claim 1, wherein the thermostable mismatch repair protein(s) comprises a MutL homologue, a MSH2 homologue, a MSH6 homologue, a MutM homologue, a MutY homologue, a MutT homologue, a MutH homologue, a HexA homologue, a HexB homologue, or a GTBP/p160 homolog.

12. (Amended.) The method of claim 1, wherein the denaturing is achieved by increasing the temperature of the solution, preferably to at least 90°C.

14. (Amended.) The method of claim 1, wherein steps b) through d) are repeated for between 1 and 10 cycles; wherein the new duplexes of step d) serve as new template polynucleotides in step b) in each subsequent cycle.

15. (Amended.) The method of claim 1, wherein steps b) through d) are repeated for at least 10 cycles; wherein the new duplexes of step d) serve as new template polynucleotides in step b) in each subsequent cycle.

16. (Amended.) The method of claim 1, wherein additional steps are performed, said additional steps comprising:

- f) generating a gene library by cloning the plurality of recombined polynucleotides;
- g) expressing and screening the gene library for an activity or property of interest; and
- h) isolating or identifying the recombined polynucleotide which gives rise to the activity or property of interest.

17. (Amended.) A plurality of recombined polynucleotides generated by a method as defined in claim 1.

18. (Amended.) A recombined polynucleotide generated by a method as defined in claim 1.

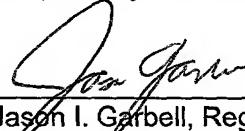
## REMARKS

This amendment is submitted to cancel claims in order to reduce the filing fee. There is no new matter added, and entry of the amendment is respectfully requested.

This application contains a Sequence Listing. Applicants enclose a computer-readable form of the Sequence Listing. The content of the paper copy of the Sequence Listing and of the computer readable form is the same.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

  
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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of: Jesper Vind Serial No.: To be assigned  
Confirmation No: To be assigned Group Art Unit: To be assigned  
Filed: December 4, 2001 Examiner: To be assigned  
For: Method For Producing a Polynucleotide Library

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

Sir:

Below is a marked-up version of the amendments made in the accompanying amendment.

## IN THE CLAIMS:

The claims have been amended as follows:

1. (Unchanged.) A method for forming a plurality of recombined homologous double-stranded polynucleotides from at least two homologous double-stranded template polynucleotides, said method comprising the steps of:

- a) providing a solution comprising at least two non-methylated homologous double-stranded template polynucleotides and one or more mismatch repair protein(s);
- b) denaturing the template polynucleotides into single-stranded polynucleotides;
- c) annealing the different single-stranded polynucleotides, wherein heteroduplexes are formed;
- d) allowing the mismatch repair protein(s) to repair nucleotide mismatches in the heteroduplexes, wherein recombined new duplexes are formed; and
- e) optionally, repeating steps b) through d) for one or more cycles; wherein the new duplexes of step d) serve as new template polynucleotides in step b) in each subsequent cycle.

2. (Unchanged.) The method of claim 1, wherein the at least two homologous double-stranded template polynucleotides are obtained by PCR amplification.

3. (Unchanged.) The method of claims 1 or 2, wherein the at least two homologous double-stranded template polynucleotides encode homologous polypeptides.

4. (Amended.) The method of [any of claims 1 – 3] claim 1, wherein the at least two homologous double-stranded template polynucleotides encode homologous enzymes, preferably amylases, proteases, cellulases, lipases, xylanases, or phospholipases.

5. (Amended.) The method of [any of claims 1 – 4] claim 1, wherein the solution comprises a population of cells or a lysate of a population of cells.

6. (Unchanged.) The method of claim 5, wherein the population of cells or the lysate of a population of cells comprises the at least two homologous double-stranded template polynucleotides.

7. (Amended.) The method of [claims 5 or 6] claim 5, wherein the population of cells or the lysate of a population of cells comprises the mismatch repair protein(s).

8. (Amended.) The method of [any of claims 5 – 7] claim 5, wherein the population of cells, or the population of cells giving rise to the lysate, do not methylate newly synthesized polynucleotides.

9. (Amended.) The method of [any of claims 1 – 8] claim 1, wherein the mismatch repair protein(s) is (are) thermostable.

10. (Amended.) The method of [any of claims 1 – 9] claim 1, wherein the thermostable mismatch repair protein(s) comprises a MutS homologue, preferably MutS YT1 of *Thermus aquaticus*.

11. (Amended.) The method of [any of claims 1 – 9] claim 1, wherein the thermostable mismatch repair protein(s) comprises a MutL homologue, a MSH2 homologue, a MSH6 homologue, a MutM homologue, a MutY homologue, a MutT homologue, a MutH homologue, a HexA homologue, a HexB homologue, or a GTBP/p160 homolog.

12. (Amended.) The method of [any of claims 1 – 11] claim 1, wherein the denaturing is achieved by increasing the temperature of the solution, preferably to at least 90°C.

13. (Unchanged.) The method of claim 12, wherein the annealing is achieved by lowering the temperature of the solution, preferably at least to a temperature at which the mismatch repair protein(s) functions, more preferably at least to between 55°C and 75°C.

14. (Amended.) The method of [any of claims 1 – 13] claim 1, wherein steps b) through d) are repeated for between 1 and 10 cycles; wherein the new duplexes of step d) serve as new template polynucleotides in step b) in each subsequent cycle.

15. (Amended.) The method of [any of claims 1 – 13] claim 1, wherein steps b) through d) are repeated for at least 10 cycles; wherein the new duplexes of step d) serve as new template polynucleotides in step b) in each subsequent cycle.

16. (Amended.) The method of [any of claims 1 – 15] claim 1, wherein additional steps are performed, said additional steps comprising:

- f) generating a gene library by cloning the plurality of recombined polynucleotides;
- g) expressing and screening the gene library for an activity or property of interest; and
- h) isolating or identifying the recombined polynucleotide which gives rise to the activity or property of interest.

17. (Amended.) A plurality of recombined polynucleotides generated by a method as defined in [any of the claims 1 – 16] claim 1.

18. (Amended.) A recombined polynucleotide generated by a method as defined in [any of the claims 1 – 16] claim 1.